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ANNUAL REPORT

Project Number	*TA-MOU-97-C17-002*
Period covered	February 1 1998 – January 31, 1999
Entire project period	February 1, 1998 – January 31, 2001
Principal Investigators	A Warburg (Israel), Y T Touré (Mali)

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SECTION 1

A Research Objectives

To identify potential aestivation habitats for *Anopheles gambiae*, evaluate their importance as sources for rainy season "recolonization" and the subsequent resumption of malaria transmission Results of field studies will also clarify possible aestivation by other important malaria vectors, most notably *An funestus* The following subsidiary objectives were undertaken to achieve this

1 Devise methods for capturing mosquitoes entering or exiting potential aestivation shelters Devise methods for extracting aestivating mosquitoes from different types of shelters Measure the gas composition and temperature inside rodent burrows and other aestivation sites of *An gambiae*

2 Characterize populations of aestivating mosquitoes or those attempting to enter or exit shelters during different times of the year in terms of their karyotype, gonotrophic status and *Plasmodium* sporozoite infection rates

3 Simulate in the laboratory, putative environmental cues for aestivation and study their effects on the physiology of mosquitoes (metabolic rate, gonotrophic cycles, and longevity)

B Research Accomplishments - Mali

Field studies were initiated during the dry season (March 1998) in the village of Doneguebougou, some 25-km NE of Bamako In order to monitor and compare spatial and temporal differences in populations of *An gambiae* s l , collections of mosquitoes were also initiated in 3 additional villages, Banambani (10km W of Doneguebougou), Kalaban Coro (E of Bamako) and Moribabougou (W of Bamako)

B 1 Summary of Results

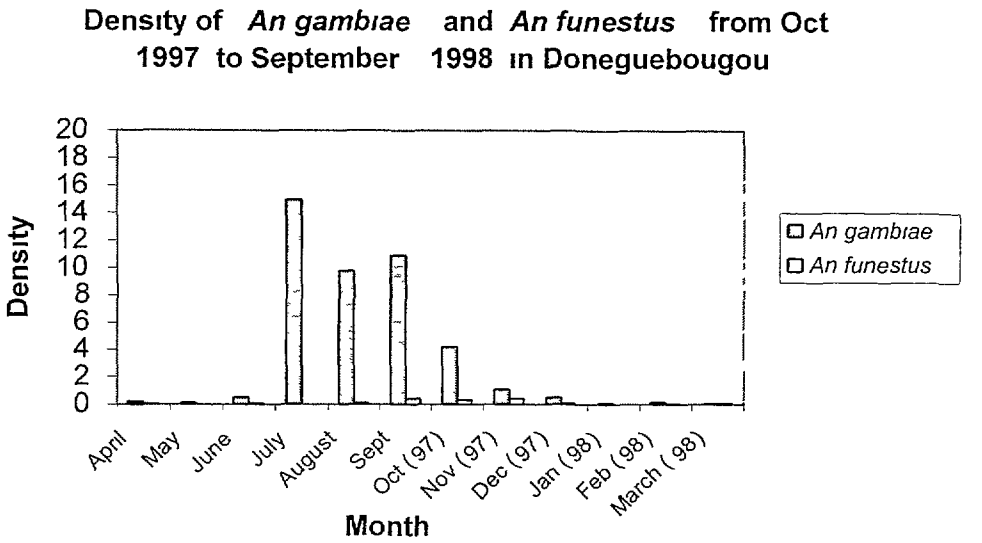
B 1 1 Spray catch sampling (30 bedrooms/sample)

These were performed in order to monitor presence of *An gambiae* and *An funestus* in the villages during the dry months Results confirmed that villages situated close to permanent bodies of water (for example Kalaban Coro) have more mosquitoes than those that are far away from these sources (e g Doneguegougou)[Table 1] Based cumulated data [Fig 1] we concentrated our efforts in Doneguebougou, which is the least affected by continued mosquito breeding in the dry season

Table 1 Spray catches in houses in 4 villages in the vicinity of Bamako
Months *March/April /May 1998*

	An gambiae s l					*An funestus*				
Village	**J**	**Gor**	**S gr**	**Gr**	**Total**	**J**	**Gor**	**S gr**	**Gr**	**Total**
Doneguebougou	1	3	16	24	**47**	0	1	8	7	**16**
Banambani	5	25	45	78	**153**	0	0	2	6	**8**
Kalaban Coro	77	28	389	302	**796**	0	0	2	5	7
Moribabougou	28	23	148	168	**367**	2	3	23	40	**68**

Figure 1 Density of mosquitoes/ room / sampling



B 1 2 Summary of Anopheline catches in Doneguebougou Sites other than bedrooms in human compounds in, April – Sept 1998

Putative aestivation sites were selected and monitored during the dry season and transition into rainy season of 1997

i) *Rodent burrows* Twenty-five rodent burrows along transect lines to the north and east of the primary field site at Doneguebougou, were identified as putative refuges for aestivating mosquitoes These were and are currently being sampled for exiting mosquitoes using inverted funnel exit traps Insects are retained in paper cans, which are inspected each morning

ii) *Termite mounds* Emergence cages have been placed over five termite mounds, ranging in size from 2x3m to 4x4m Traps are sampled on a daily basis every morning (dry and transition seasons) Anopheline mosquitoes have not been recovered from termite mounds although an increase in numbers of insects within the cages (predominantly Hymenoptera) has been noted during the transition from dry to wet season

iii) *Tree hollows/holes* All trees within an 800m radius from the center of Doneguebougou have been inspected for tree holes Some thirty trees were identified and sampled

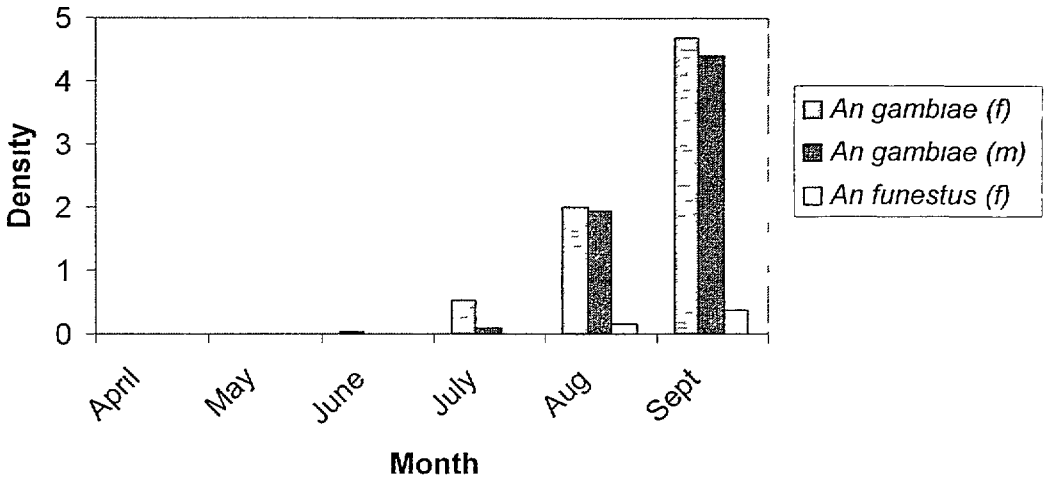
iv) *Human bait landing catches* Night catches of mosquitoes landing on human collectors were made during the transition period from dry to wet season Collections were made in the outlying bush close to dry larval breeding sites Simultaneous sampling at two houses in the village allowed comparison of catches between the village and bush

Anopheline catches in the following graphs are expressed in population density = The total number of mosquitoes per individual site, per month Numbers are average

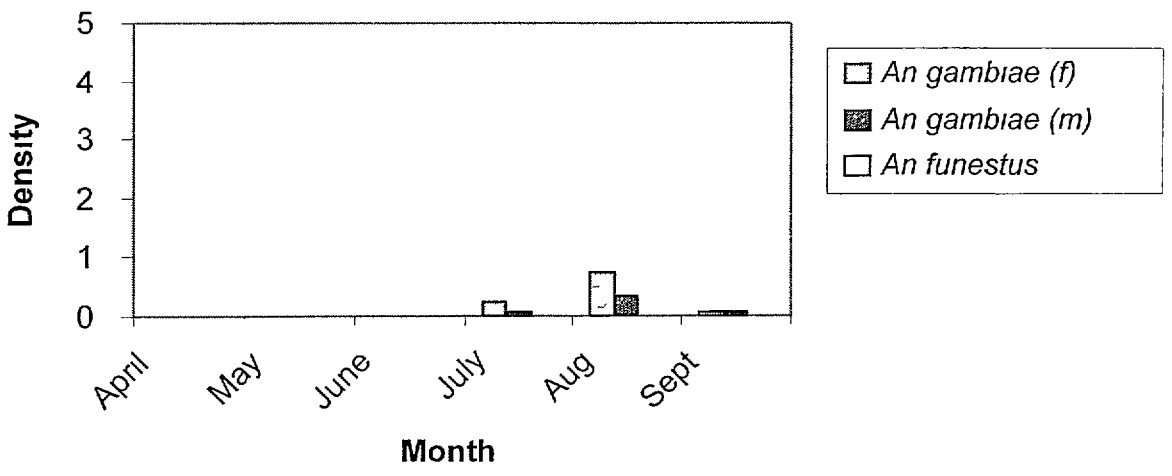
1 Anopheline density in animal enclosures, April - Sept 1998

Anopheline catches in the following graphs are expressed in population density = The total number of mosquitoes per individual site, per month Numbers are average for all sites sampled Catches were made with aspirators

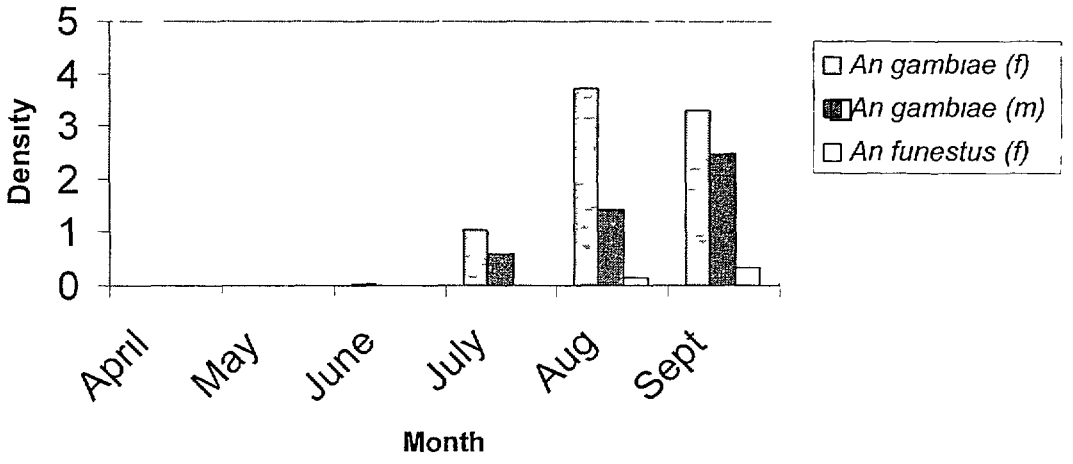
1 Anopheline density in animal enclosures, April - Sept 1998



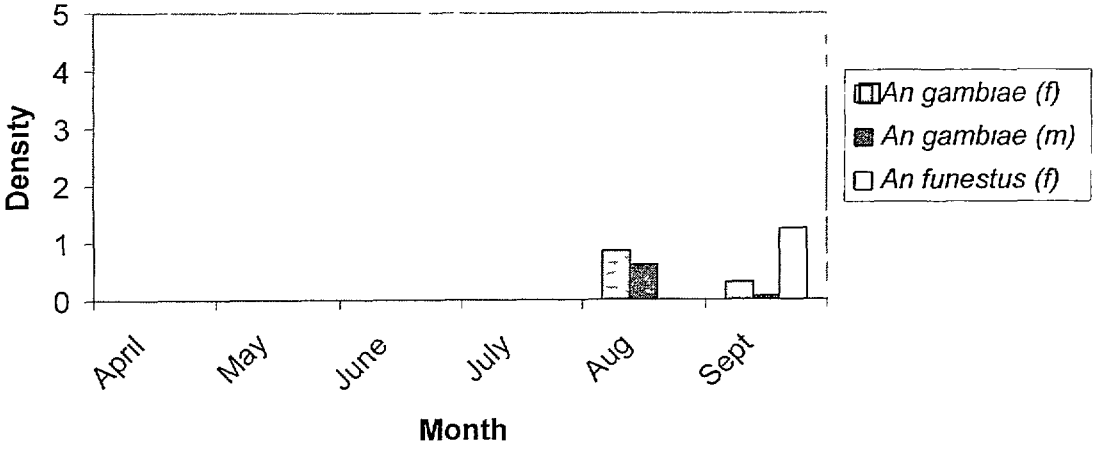
2 Anopheline density in chicken coops, April - Sept 1998



3 Anopheline density in abandoned houses April - Sept 1998



4 Anopheline density in tree holes/termite mounds April - Sept 1998



B 1 3 Summary – Mali

Thus far we have failed to identify large concentrations of aestivating mosquitoes Several reasons may account for this the most likely being that inactive mosquitoes are very hard to trap We are continuing the monitoring this year with several additional approaches

1 We shall make use of insect flight traps to monitor any movement of mosquitoes from putative aestivation sites to the village during the transition from dry to rainy season
2 We have purchased large mechanical aspirators in order to sample larger shelters such as caves and caverns for presence of mosquitoes in the dry season
3 We shall perform more comparative human bait catches between putative aestivation sites and the village

B 2 Research Accomplishments – ISRAEL

We perfected a system to measure temperature dependent changes in metabolism and the energetics of ovarian development of two mosquito species, *Aedes aegypti* and *Anopheles gambiae* A general characteristic of diapause is reduced metabolism We presume that *An gambiae* mosquitoes aestivating in the dry months will be in a state of lowered metabolic rates and are currently investigating ways to characterize this and measure its various components

A flow - through system was developed to measure rates of both O_2 consumption and CO_2 production By monitoring both O_2 and CO_2 levels, a more accurate representation of energy budgets and substrate utilization is gained without exposing insects to caustic or dehydrating vapors The effects of temperature on the resting metabolism of *Anopheles gambiae* Giles are reported on Additionally we describe changes in the metabolic rate prior to and following blood feeding, through the ovarian cycle and oviposition

B 2 2 Experimental procedures and results

An gambiae (G3) were reared from an established colony at the Department of Parasitology, Hebrew University Larvae were raised at a density of 100 per 500ml water and fed a mixture of cat food and fish flakes Pupae were removed and placed in individual cages for emergence every 12 hr in order to maintain a known age class Adults were allowed unlimited access to 10% sucrose solution Both adults and larvae were maintained at 28°C, 70% RH (+5%) and under a 16 8 photoperiod When necessary, adult females were blood feed on a human volunteer on day 6, post emergence

Flow – through respirometry VO_2 and VCO_2 were measured continuously from groups of 5 mosquitoes using an open-flow respirometry system The respirometry chambers comprised 2-ml glass syringes supplied with dry air from a Brooks Series 5850 mass-flow controller Chambers were placed in a small, constant temperature water bath during all measurements, temperatures were maintained at $\pm$ 0 5°C Flow rates into the chamber were maintained at 2 ml/min, corrected for standard temperature and pressure (STP) Excurrent air was re-dried and fed first to a Servomex Series 1400 CO_2 analyzer and then to an Applied Electrochemistry S-3A O_2 analyzer The analyzers

were zeroed against N_2 and referenced, at the start and end of experiments, against both incurrent air and an air mix comprising 0 83 % CO_2 /19% O_2

Protocol Females, whether blood fed or not, at seven days post emergence were used as the starting point from which all experiments proceeded In order to prevent prior exposure to CO_2, female mosquitoes were only placed within respirometry chambers after calibration of the analyzers and were allowed a 30 min acclimatization period VO_2 and VCO_2 were subsequently recorded during a one-hour period and resting metabolism was assessed by taking a five-minute sample during which VO_2 was minimal

Data analysis Workbench data files were transferred to Excel for calculation of VO_2 and VCO_2 All values of metabolic data in the text are given as means (±SE) Analyses were performed using the statistical software package Statistica, Version IV A polynomial regression was used to test the significance of the relationship between temperature and VO_2 for both *Ae aegypti* and *An gambiae* and within species differences analyzed by ANCOVA Differences in VO_2 between species at each temperature were compared by t-test ANOVA was used to test the significance of any differences in RQ values across the temperature range

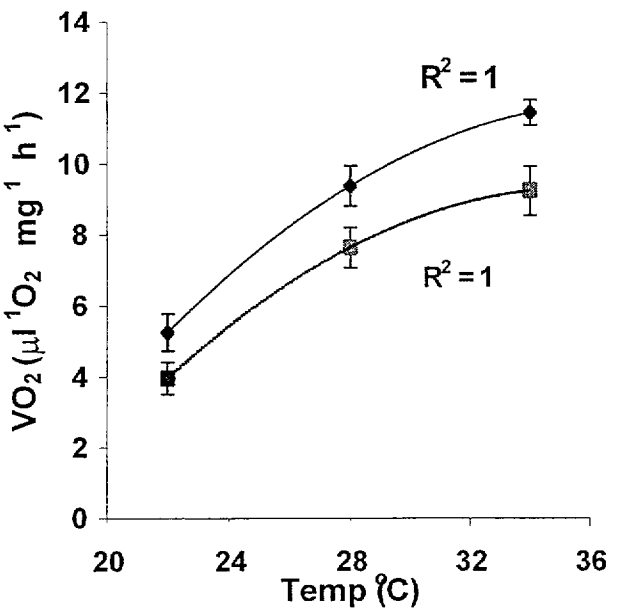


Figure 1 The relationship between mean VO_2 (±SE) and temperature of *Aedes aegypti* (closed diamonds) and *Anopheles gambiae* (closed squares) Each point represents the mean value from 10 replicates Polynomial regression lines are fitted to the values as a solid line (*Ae aegypti*) and dashed line (*An gambiae*)

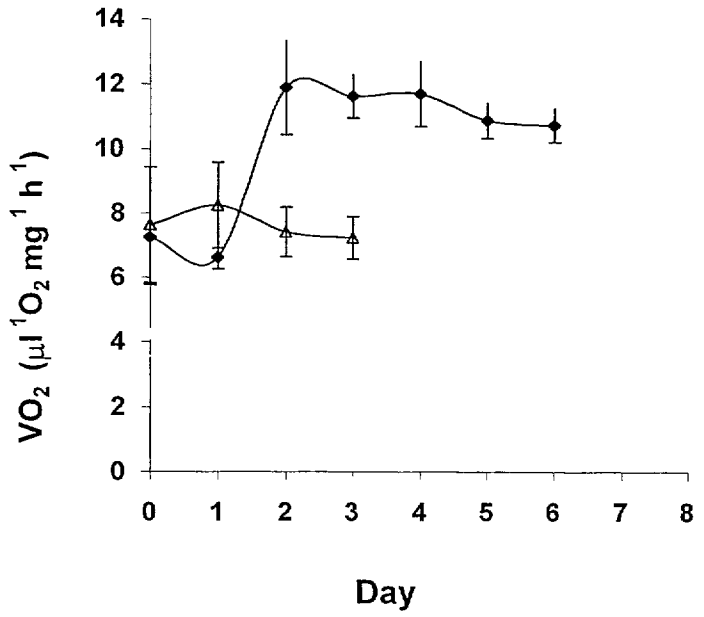


Figure 2 Patterns of mass specific VO2 (±SE) of a) *Aedes aegypti* and b) *Anopheles gambiae* maintained on a 10% sucrose diet (open triangles, solid line), after blood feeding (closed diamonds, solid line) and following oviposition (closed triangles, dashed line) Each point represents the mean value from 10 replicates Day zero represents insects at day 7-post emergence when, in the case of blood fed individuals, they were allowed access to a blood meal

B 2 3 Summary and future studies

The flow-through, continued measurement system is functioning consistently and satisfactorily For the first time, the ratio between temperature increase and respiratory rates can be analyzed Furthermore, results indicate that blood feeding increases metabolic rates and oviposition causes a temporary reduction The sensitivity of the system will allow us to determine lowered metabolism of even small numbers of mosquitoes Future studies will include determination of metabolic rates of *An gambiae* mosquitoes maintained under conditions favoring aestivation

C Scientific Impact of Collaboration

a) Visit of Israeli team to Mali

Immediately following the approval of funding by CDR, on March 3rd 1998, A Warburg and A Bhasin (Postdoctoral fellow) traveled to Mali The visit proved very productive Both conceptual and technical discussions were held and 3 field trips were carried out On 5/3/98 we went to Banambani and on 6/3/98 to Doneguebougou These were preliminary visits to allow the Israeli team to familiarize with the study sites and select the principal study village Following these trips it was decided to focus primarily on Doneguebougou and concentrate on caverns, rodent burrows, tree holes and termite mounds as possible aestivation sites Dr Bhasin remained in Mali until April 12th 1998 to coordinate and participate in the ensuing field work with A, Dao During this period two, lengthy field studies were conducted in Doneguebougou

On May 19th 1998, Dr Bhasin returned for another working visit lasting till 17th June He made a presentation describing his work on respiratory physiology being conducted in Israel

And participated in two additional field trips with A Dao and the team (23 - 28/5/98 Field trip 1, Doneguebougou & Banambani 3/6 -14/6/98 Field trip 2, Doneguebougou & Banambani He also coordinated the efforts to collate and analyze relevant data As of March 28 Dr Bhasin has returned to Mali He will stay there through May (end of dry season) to coordinate and participate in fieldwork as detailed above (section B 1 3)

a) Visit of Adama Dao to Israel A Dao, the Malian scientist coordinating the aestivation studies worked in Israel for 7 weeks during September and October 1998 He participated with Dr Bhasin in the respiratory physiology study being conducted in Tel Aviv under the supervision of Prof A Ar He and Dr Bhasin also devoted time to summarize all data collected in Mali and formulated the plans for future field studies A Dao also worked in Dr Warburg's laboratory where he learned some of the techniques relevant to parasite culture and mosquito-parasite interactions A Dao's visit proved very useful and has contributed both scientifically and socially to the continued collaborative efforts of both teams

D **Description of Project Impact**

The main impact of the project has been to prioritize the study of mosquito physiology in general and estivation in particular, as important "unknowns" in the study of the transmission epidemiology of malaria in sub-Saharan Africa Ours is the only project, Worldwide, emphasizing these topics

E **Strengthening of developing country institutions**

The techniques required for open-flow respirometry have not been applied to the study of malaria vectors Once basic procedures have been established and preliminary date obtained the entire continuation will be carried out in Mali Training of Adama Dao and the close interaction of Dr Bhasin with the entire Malian team are laying the foundation for this transition

F **Future work**

This annual report covers the first year of work but only 1 dry season As stated above we plan to continue and expand our efforts as listed in the objectives section and detailed above (sections B 1 3 and B 2 3)

SECTION 2

A **Managerial issues** None

B **Budget** None

C **Special Concerns** None

D **Collaboration, Travel, Training & Publications**

See Section 1 C page 9-10 A manuscript on respiration studies is in preparation

E **Request for Tel Aviv AID actions** N/A